

FLUORIDE ION REGENERATION OF CYCLOSARIN (GF) FROM RAT BLOOD FOLLOWING WHOLE-BODY EXPOSURE TO LETHAL LEVELS OF GF VAPOR

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ABSTRACT

A sensitive and selective method has been developed for a cyclohexyl methylphosphonofluoridate (GF) nerve agent biomarker in tissue and biological fluids after GF lethal level whole body inhalation exposure. Levels of exposure ranged from 2 mg/m³ for 240 min to 41.9 mg/m³ for 10 min. The GF biomarker found in rat plasma and red blood cell samples was regenerated GF, which is the product of adding fluoride ion at pH 4 to the post exposure samples. Regenerated GF was separated from the biological matrix using a C18 solid-phase extraction (SPE) sample preparation. Samples are concentrated by injecting 2-200 uL of ethyl acetate extract on a Tenax[®]-TA sorbent tube along with 200 pg of decadeuterated diethyl ethyl phosphonate as the internal standard followed by thermal desorption GC-FPD analysis and GC-MS confirmation. The method detection limit was approximately 10 pg of agent on column. Quantities of regenerated GF found in plasma samples ranged from 230 to 572 ng/g. Red blood cell samples from the same animals contained considerably less biomarker ranging from 1.45 to 30.0 ng/g. In cases where animals died just after the exposure was complete and post mortem blood could be collected the levels of regenerated GF in both plasma and red blood cells were only 60 % of the amount found in the living rats.

OBJECTIVES

Investigate Possible Dosemetrics:

- Develop internal dose methods
- Extend regenerated GB (R-GB) assay to GF (R-GF)
- Support PBPK modeling effort

Rat:

- Basic Toxicological Model
- Compare to GB data from similar exposure experiments.
- Post mortem vs. survivor samples

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MATERIALS

- GF&GB : CASARM grade prepared and analyzed at ECBC and diluted with hexane.
- Decadeuterated diethyl ethylphosphonate (2H10DEEP): Synthesized at ECBC
- C18 SPE cartridges: 200mg (Waters Associates, Millipore Corp., Milford, MA)
- Acetate buffer (pH3.5)
- Potassium fluoride: 6 M
- Chemicals: All other chemicals were procured commercially at ACS reagent grade or higher.

SAMPLES

Inhalation Exposure Samples

Whole blood from GF exposed rats was collected with EDTA via tail vein (survivors) or by heart stick (post mortem) after inhalation exposure. Samples were centrifuged at 15,000 rpm for 3 min. The resulting red blood cell pack and serum/plasma samples were analyzed for regenerated agent by the addition of acetate buffer and fluoride ion.

Sample Preparation

- 1) Weigh Sample (0.1-0.5 g).
- 2) Add and mix(vortex): 1.5 mL acetate buffer pH 3.5, and 0.02 mL(for plasma) or 0.2 mL (for packed cells or tissue) of KF solution.
- 3) Centrifuge 15000 rpm for 3 min (RBC & tissue samples).
- 4) Transfer liquid to conditioned C18 SPE column. Columns conditioned with 1 mL ethyl acetate, 1 mL isopropanol, and finally 1 mL acetate buffer.
- 5) Elute with 1 mL ethyl acetate over sodium sulfate
- 6) Spike 0.010-0.200 mL ethyl acetate extract on DAAMS tube (Tenax-TA), and then spike tube with internal standard (400 pg of 2H10-DEEP), flush with N₂ for 3-6 min at 75 cc/min

METHODS & ANALYSIS

- Sample Introduction: Dynatherm ACEM 900 (Dynatherm Inc., Kelton, PA) Tube:200°C/3min, Trap: 265°C/3min, Valve:230°C, Interface: 250°C
- GC Column: DB5-30 m x 0.25 mm x 0.5 mm film
- GC Oven program: Initial Temp 35 °C(3 min) to 140 °C @ 150 °C/min (0 min hold) to 265°C @50 °C/min(2.3 min hold)
- DAAMS Tube: Reconditioned at 275°C for 4-6 min
- Detection: Dual Flame Photometric Detectors or MSD (Model 6973 MSD Agilent, Avondale, PA)

RESULTS

- Method detection limit for R-GF was approximately 10 pg of agent on column
- Quantities of R-GF found in plasma samples ranged from 230 to 572 ng/g, shown in Figures 1, 2, &3.
- Red blood cell samples from the same animals contained considerably less biomarker ranging from 1.0 to 120 ng/g.

- In 10 min exposure cases where animals died just after the end of exposure and postmortem blood could be collected, the levels of R-GF in both plasma and red blood cells were only 60 % of the amount found in the survivors, Figure 4.
- Postmortem R-GB levels were only 40-50 % of the survivors, Figure 4.
- Miosis level GF exposure yielded R-GF which could be correlated to exposure levels, Figure 5.

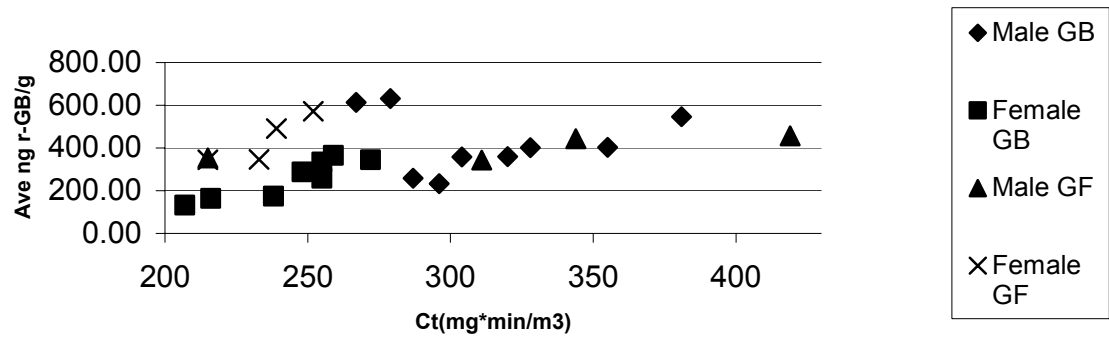


Figure 1. Plasma R-GB vs R-GF: 10 Min Exposure.

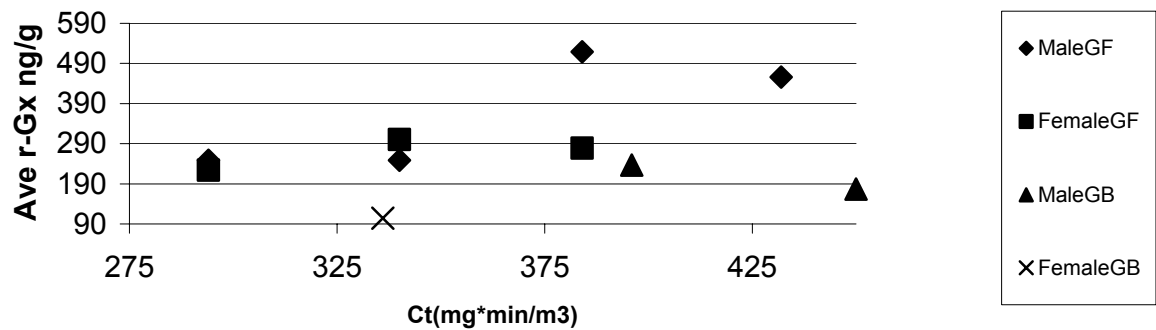


Figure 2: Plasma R-GB vs R-GF: 60 Min Exposure.

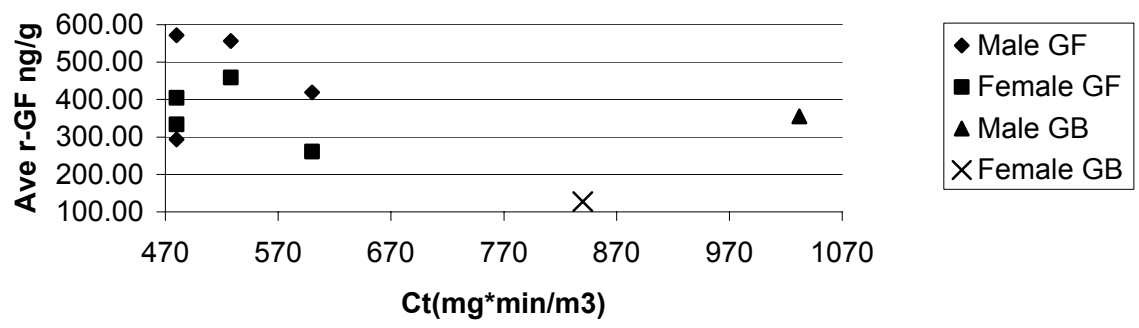


Figure 3. Plasma R-GB vs R-GF: 240 Min Exposure.

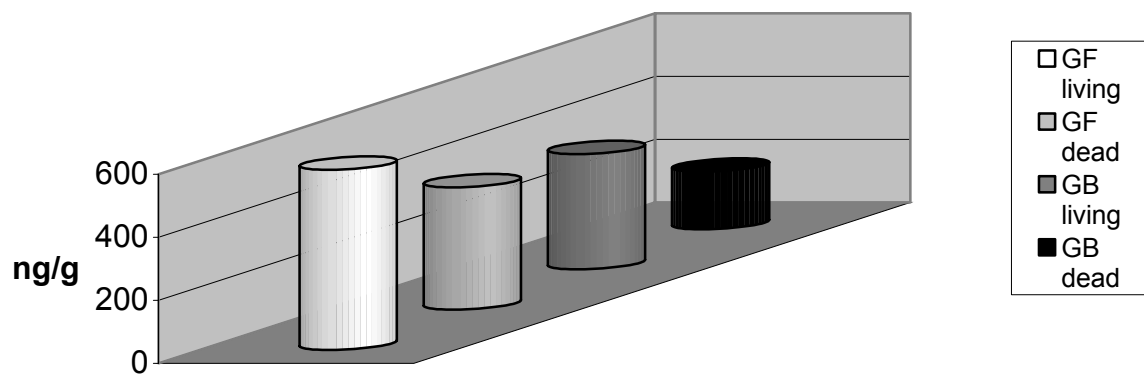


Figure 4: Plasma Levels of R-GB/GF:10 min Exposure @ Approximately 250 mg*min/m3

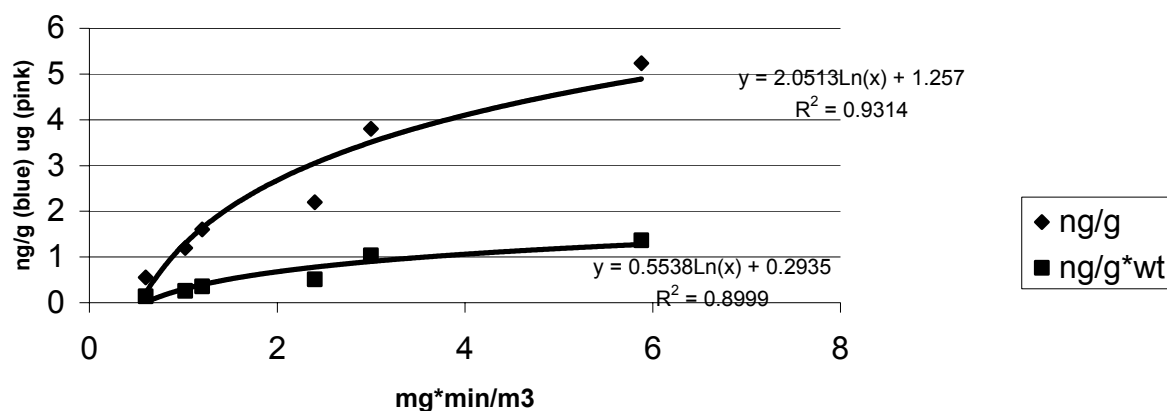


Figure 5: Miosis Level Ct vs Recovered R-GF in Rat Plasma: 60min Exposure

CONCLUSIONS

- Developed potential dosemetric assay to assess internal dose of GF in rat blood samples resulting from vapor exposure
- Preliminary studies of lethal and miosis GF vapor inhalation exposure in rats were completed:
- Plasma R-GF levels much greater than RBC levels
- Plasma levels drop sharply after death
- 10 and 60 min lethal level GF exposures yielded amounts of R-GF comparable to 10 and 60 min lethal GB exposures
- Miosis levels could be assessed and appear to correlate to exposure.